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(54) Title: RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

(57) Abstract

The cDNA that encodes a glycoprotein receptor from the tobacco hornworm which binds a *Bacillus thuringiensis* toxin has been obtained and sequenced. The availability of this cDNA permits the retrieval of DNAs encoding homologous receptors in other inserts and organisms as well as the design of assays for the cytotoxicity and binding affinity of potential pesticides and the development of methods to manipulate natural and/or introduced homologous receptors and, thus, to destroy target cells, tissues and/or organisms.

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RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

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Technical Field

The invention relates to receptors that bind toxins from *Bacillus thuringiensis* and thus to pesticides and pest resistance. More particularly, the invention concerns recombinantly produced receptors that bind BT toxin and to their use in assays for improved pesticides, as well as in mediation of cell and tissue destruction, dissociation, dispersion, cell-to-cell association, and changes in morphology.

Background Art

It has long been recognized that the bacterium *Bacillus thuringiensis* (BT) produces bacteriocidal proteins that are toxic to a limited range of insects, mostly in the orders Lepidoptera, Coleoptera and Diptera. Advantage has been taken of these toxins in controlling pests, mostly by applying bacteria to plants or transforming plants themselves so that they generate the toxins by virtue of their transgenic character. The toxins themselves are glycoprotein products of the *cry* gene as described by Höfte, H. *et al.* Microbiol Rev (1989) 53:242. It has been established that the toxins function in the brush border of the insect midgut epithelial cells as described by Gill, S.S. *et al.* Annu Rev Entomol (1992) 37:615. Specific binding of BT toxins to midgut brush border membrane vesicles has been reported by Hofmann, C. *et al.* Proc Natl Acad Sci USA (1988) 85:7844; Van Rie, J. *et al.* Eur J Biochem (1989) 186:239; and Van Rie, J. *et al.* Appl Environ Microbiol (1990) 56:1378.

Presumably, the toxins generated by BT exert their effects by some kind of interaction with receptors in the midgut. The purification of a particular receptor from *Manduca sexta* was reported by the present inventors in an article by Vadlamudi, R.K. *et al.* J Biol Chem (1993) 268:12334. In this report, the receptor protein was isolated by immunoprecipitating toxin-binding protein complexes with toxin-specific antisera and separating the complexes by

SDS-PAGE followed by electroelution. However, to date, there has been no structural information concerning any insect receptor which binds BT toxin, nor have, to applicants' knowledge, any genes encoding these receptors been recovered.

Disclosure of the Invention

The invention provides recombinant materials for the production of BT toxin-binding receptors as well as methods to employ these materials to generate receptors for use in screening assays for candidate pesticides. Since the native cDNA sequence encoding this receptor, designated BT-R_1, has been retrieved from the tobacco hornworm, encoding DNA for receptors in other species of insects, as well as in other organisms, which have homology to hornworm receptor can be obtained.

Thus, in one aspect, the invention is directed to a polynucleotide in purified and isolated form which comprises a nucleotide sequence encoding a receptor that binds a BT toxin and other ligands and which has the requisite homology to the BT-R_1 protein.

In other aspects, the invention is directed to expression systems for nucleotide sequences encoding the receptor, to methods of producing the receptor recombinantly, to the receptor as thus produced, to antibodies specifically immunoreactive with the receptor, to assay methods useful for screening candidate pesticides, to antisense polynucleotides corresponding to the coding sequence, to methods of targeting tissues and/or cells using the binding characteristics of the receptor, and to methods of manipulating tissues and/or cells using the function of the receptor.

Brief Description of the Drawings

Figure 1 shows the nucleotide sequence and deduced amino acid sequence of cDNA encoding the BT-R_1 protein from *M. sexta*.

Figure 2 shows a comparison of amino acid sequences of cadherin motifs (BTRcad-1 to 11) in BT-R_1 to those of other cadherins.

Modes of Carrying Out the Invention

The invention provides, for the first time, sequence information concerning receptors that bind BT toxins in insect midguts.

The BT-R_1 cDNA clone retrieved as described in the examples below encodes a protein having an identical amino acid composition with that described for the native receptor. Furthermore, toxin-binding specificity and immunoreactivity are similar. The native 210 kD BT-R_1 specifically recognizes *cryIA(b)* toxin of BT-*berliner*; a K_d value of 708 pM was obtained for the native protein.

The *cryIA(b)* toxin selectively kills *M. sexta* larvae with an LC_{50} of 7.5 ng/cm^2 of diet surface. BT-R_1 binds the toxin under both reducing and nonreducing conditions and protease treatment of intestinal BBMV vesicles prepared from *M. sexta* showed that a 50 kD fragment of the 210 kD receptor is sufficient for toxin binding. The 50 kD toxin-binding domain is extracellular since the intestinal BBMV vesicles are oriented predominantly right side out as reported by Haase, W.H. *et al.* Biochem J (1978) 172:57. This is consistent with the characteristics of the deduced amino acid sequence of the cDNA clone described below, as well as with the binding of toxin to the surface of intact BT-R_1 transfected human embryonic 293 cells as described in Example 3.

Whereas a particular cDNA clone from the tobacco hornworm has been described as illustration, the availability of this sequence information permits retrieval of corresponding receptors responsive to BT and related toxins from other species. This is conveniently accomplished by using the cDNA obtained in the present invention as a probe for screening cDNA or genomic libraries under conditions of stringency which eliminate false positives and retrieve substantially only corresponding receptors with coding sequences that are homologous to the coding sequence for the receptor of the present invention. Thus, the BT-R_1 protein itself and receptor proteins encoded by a nucleotide sequence homologous to the native nucleotide sequence encoding BT-R_1 are provided by the invention. Alternatively, PCR-mediated cloning can be used; however, this method does not take advantage of the detailed and complete information that resides in the availability of the nucleotide sequence encoding the full-length receptor from *M. sexta*. Also, PCR-mediated cloning introduces

errors in natural DNA sequences. Thus, by using the full-length cDNA as a probe under conditions of appropriate stringency, only nucleotide sequences encoding the corresponding receptors will be obtained. The standard hybridization conditions include hybridization with nonspecific DNA such as salmon DNA at 500C and washing at 450C. To obtain corresponding receptors having the lowest detectable homology with the receptor from *M. sexta*, the cDNA probe is hybridized under conditions of low standard stringency (30-370C and 4-6X SSC. More closely related corresponding receptors are obtained by hybridizing the cDNA probe under moderate standard stringency conditions (40-500C in 1X SSC).

The distribution of receptors of appropriate homology in the animal kingdom is believed to be fairly wide. Indeed, it is thought that higher organisms such as mammals, including primates, contain corresponding receptors which are homologous to BT-R_1 but respond to modified forms of BT toxins. In addition, other parasites such as nematodes, both those that afflict plants and those that afflict animals, will contain corresponding receptors.

Although one of the advantages of the use of BT toxins as insecticides is its specificity for certain orders of insects, this specificity is believed to result from the particular structure of the BT toxin rather than the unavailability of a corresponding mechanism in other insect orders. Thus, modified forms of BT toxin would be effective with respect to insects which contain homologous but slightly different forms of the receptor from that of the BT-R_1 protein illustrated below.

As used herein, "A receptor that specifically binds a BT toxin" refers to a receptor which is homologous to the BT-R_1 protein illustrated herein and which binds to either BT toxins themselves or to BT toxins that are sufficiently modified so as to bind these receptors which provide the required homology to BT-R_1.

The criteria for inclusion of a receptor in the present invention are the requirements that 1) it behave as a receptor -- i.e., be capable of being displayed at the cell membrane; 2) it be sufficiently homologous to the BT-R_1 receptor described herein that a nucleotide sequence encoding the protein hybridizes under the stringency conditions described above to the nucleotide sequence encoding BT-R_1 as shown in Figure 1; and 3) when displayed on the surface of a cell, it is capable of binding a BT toxin or a modified form of BT toxin that exerts

a cytotoxic effect either on the cell in which the receptor resides or in a tissue with which the cell is associated.

The structural characteristics of the "modified BT toxin" are defined by the functional property set forth above, but it may be convenient to design modified forms of BT toxin by conservative amino acid substitutions or other known protein-manipulating techniques applied to naturally occurring BT toxins.

The presence of similar receptors in noninsect organisms as well as other insects besides those harboring BT-R_1 is supported by the sequence similarity of the BT-R_1 protein to that of the various members of the cadherin superfamily of proteins, which are membrane glycoproteins believed to mediate calcium-dependent cell aggregation and sorting. See, for example, Takeichi, M. Science (1991) 251:1451; and Takeichi, M. N Rev Biochem (1990) 59:237.

Included in this superfamily are desmoglien, desmocollins, the *Drosophila fat* tumor suppressor, human intestinal peptide transport protein and T-cadherin. All of these proteins share common extracellular motifs although their cytoplasmic domains differ. Goodwin, L. *et al.* Biochem Biophys Res Commun (1990) 173:1224; Holton, J.L. *et al.* J Cell Sci (1990) 97:239; Bestal, D.J. J Cell Biol (1992) 119:451; Mahoney, P.A. *et al.* Cell (1991) 853; Dantzig, A.H. *et al.* Science (1994) 264:430; and Sano, K. *et al.* EMBO J (1993) 12:2249. Inclusion of BT-R_1 in the cadherin superfamily is further supported by the report that EDTA decreases the binding of *cryIA(b)* toxin of BT to the 210 kD receptor of *M. sexta* (Martinez-Ramirez, A.C. *et al.* Biochm Biophys Res Commun (1994) 201:782).

It is noted below that the amino acid sequence of BT-R_1 reveals that a calcium-binding motif is present. This is consistent with the possibility that cells having receptors to bind toxin may themselves survive although they render the tissues in which they are included permeable to solutes and thus effect disintegration of the tissue. Such a mechanism is proposed for the death of insects that ingest the toxin via the epithelial cells in their midgut by Knowles, B.H. *et al.* Biochim Biophys Acta (1987) 924:509. Such a mechanism is also supported in part by the results set forth in Example 4 hereinbelow which indicate that the effect of the toxin on embryonic 293 cells modified to express the receptor at their surface is reversible.

Thus, in summary, the invention provides a family of receptors that is able to mediate the negative effects exerted by BT toxin or its modified forms on the cells expressing the receptor, by damaging the cells themselves and/or the tissue or organ of which the cells form a part. The receptor may be expressed natively at the surface of the target cells or the target cells may be modified to contain an expression system which will effect the display of receptor at the surface. The availability of this family of receptors and recombinant methods for its production and for the production of cells displaying it at their surfaces provides a number of opportunities to conduct screening assays for improved toxins, particularly insecticidal toxins, for generation of antibodies that can be useful as alternatives to chemotherapeutic agents for the destruction and/or dissociation of unwanted cells or tissues, and for the design of improved toxins and pharmaceuticals.

Screening Assays

The availability of the recombinant family of receptors of the present invention permits design of straightforward screening assays for toxins which will interact successfully with these receptors resulting in measurable effects on the cells in which the receptors reside. Briefly, suitable host cells, such as COS cells for transient expression, CHO cells for stable expression, and a variety of other mammalian and insect host cells can be modified to contain expression vectors appropriate to the hosts for the production of the receptors of the invention displayed on the surfaces of the cells. Since the receptors are natively membrane proteins, no particular design of the expression system is required in order to effect their disposition at the cell surface. Expression vectors suitable for any desired host are generally known in the art. For example, for mammalian expression, suitable control sequences include the SV40 and adenovirus promoters as constitutive promoters, the metallothionein inducible promoter, suitable enhancers, if desired, and termination signals and the like. For insect cells, the bacculovirus system is preferred. For other eucaryotic cells such as yeast, the glycolytic enzyme promoters and various amino acid synthesis promoters are commonly employed. Procaryotic cells such as *E. coli* also may be adapted for expression of the receptor in the assay of the invention, for instance by using a reporter gene under the control of cyclic AMP and operably linked to the receptor via protein G such that toxin binding will interrupt adenyl cyclase activity and thereby produce a detectable change in reporter gene activity. The assay system in a prokaryotic host may require further modification to compensate for lack of glycosylation which is known to occur in insect cells where the BT-R_1 protein is naturally expressed.

The cells are modified by transfection, retroviral infection, electroporation or other known means, to contain the desired expression system and then cultured under conditions wherein the receptor protein is produced and displayed. If desired, the cells are then recovered from the culture for use in the assay, or the culture itself can be used *per se*.

In the assays, the modified cells are contacted with the candidate toxin and the effect on metabolism or morphology is noted in the presence and absence of the candidate. The effect may be cytotoxic -- i.e., the cells may themselves exhibit one of the indices of cell death,

such as reduced thymidine uptake, slower increase in optical density of the culture, reduced exclusion of vital dyes (e.g., trypan blue), increased release of viability markers such as chromium and rubidium, and the like. The differential response between the toxin-treated cells and the cells absent the toxin is then noted. The strength of the toxin can be assessed by noting the strength of the response.

These assays may be conducted directly as described above or competitively with known toxins. For example, one approach might be to measure the diminution in binding of labeled BT cry toxin in the presence and absence of the toxin candidate.

In addition to simply screening candidates, the screen can be used to devise improved forms of toxins which are more specific or less specific to particular classes of insects as desired. The ability to determine binding affinity (K_a and K_d), dissociation and association rates, and cytotoxic effects of a candidate allows quick, accurate and reproducible screening techniques for a large number of toxins and other ligands under identical conditions which was not possible heretofore. Such information will facilitate the selection of the most effective toxins and ligands for any given receptor obtained from any desired host cell.

Competition assays may also employ antibodies that are specifically immunoreactive with the receptor. Such antibodies can be prepared in the conventional manner by administering the purified receptor to a vertebrate animal, monitoring antibody titers and recovering the antisera or the antibody-producing cells for immortalization, to obtain immortalized cells capable of secreting antibodies of the appropriate specificity. Techniques for obtaining immortalized B cells and for screening them for secretion of the desired antibody are now conventional in the art. The resulting monoclonal antibodies may be more effective than the polyclonal antisera as competition reagents; furthermore, the availability of the immortalized cell line secreting the desired antibody assures uniformity of production of the same reagent over time. The information and the structural characteristics of toxins and ligands tested will permit a rational approach to designing more efficient toxins and ligands. Additionally, such assays will lead to a better understanding of the function and the structure/function relationship of both toxin/ligand and BT-R_1 analogs. In turn, this will allow the development of highly effective toxins/ligands. Ligands include natural and modified

toxins, antibodies (anti-receptor and antiidiotypic antibodies which mimic a portion of a toxin that binds to a receptor, and whatever small molecules bind the receptors.

Therapeutic Strategies

Advantage may be taken of the ability of receptors to mediate the destruction, dissociation or association of cells, tissues or organs by utilizing the screening assay as a method to identify successful therapeutics in the treatment of, for example, malignancies, metastases and infectious microorganisms which naturally express receptors corresponding to BT-R_1. The presence of receptors corresponding to the BT-R_1 receptor illustrated herein and members of the family of receptors included in the invention in the undesired cells may be exploited by first assessing the interaction of a proposed therapeutic with the receptors on these cells in culture and then identifying agents which successfully interact with the receptors as useful candidate reagents. Antibodies reactive with these receptors comprise a class of promising therapeutic candidates.

In some applications target cells, tissues, organs, and microorganisms which do not express an effective receptor corresponding to the BT-R_1 receptor may be transformed or transfected to express an effective corresponding receptor. These targets then will be killed or manipulated with toxin or other ligands. For instance, yeast cells to be used for toxin assays for a particular insect may be transformed with a genetic construct for expression of the receptor from that insect which corresponds to the BT-R_1 receptor.

In another aspect of the invention the receptors corresponding to BT-R_1 in certain target cells may be manipulated by modified toxin or other ligands to prevent the normal response to toxin (dissociation, damage and death of membranes, cell, tissues and organisms). For instance, a ligand which binds to a corresponding receptor in such a way that normal receptor function is inhibited would thereby prevent the receptor from initiating the usual destructive effects in the presence of a normal ligand such as a toxin. In other words, the invention enables development of competitive inhibitors of a toxin or other ligand which normally initiates destructive or other effects via a receptor corresponding to BT-R_1.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Purification and Sequence Determination of BT-R_1 Protein

Midguts of *M. sexta* were extracted and the BT-R_1 protein purified according to the method of Vadlamudi, R.K. *et al.* J Biol Chem (1993) 268:1233, referenced above and incorporated herein by reference. The electroeluted band was confirmed to contain BT-R_1 protein by binding to ^{125}I-*cryIA(b)* toxin. In gel electrophoresis, the protein bound to toxin had an apparent weight of approximately 210 kD under reducing and nonreducing conditions.

The purified electroeluted BT-R_1 was subjected to cyanogen bromide digestion and the cyanogen bromide fragments separated on a 17% high-resolution tricine SDS-polyacrylamide gel as described by Schagger, H. *et al.* Anal Biochem (1987) 166:368. The separated fragments were transferred to Problott membranes (Applied Biosystems) and five bands were extracted and subjected to microsequencing using standard instrumentation. The amino acid sequences obtained were:

1. (Met)-Leu-Asp-Tyr-Glu-Val-Pro-Glu-Phe-Gln-Ser-Ile-Thr-Ile-Arg-Val-Val-Ala-Thr-Asp-Asn-Asn-Asp-Thr-Arg-His-Val-Gly-Val-Ala;
2. (Met)-X-Glu-Thr-Tyr-Glu-Leu-Ile-Ile-His-Pro-Phe-Asn-Tyr-Tyr-Ala;
3. (Met)-X-X-X-His-Gln-Leu-Pro-Leu-Ala-Gln-Asp-Ile-Lys-Asn-His;
4. (Met)-Phe/Pro-Asn/Ile-Val-Arg/Tyr-Val-Asp-Ile/Gly;
5. (Met)-Asn-Phe-Phe/His-Ser-Val-Asn-Arg/Asp-Glu.

Example 2

Recovery of cDNA

An *M. sexta* cDNA library was constructed from midgut tissue in Sgt10 using the Superscript Choice System according to the manufacturer's instructions (Life Technologies, Inc.). Degenerate oligonucleotide probes were constructed based on the peptide sequences determined in Example 1 using the methods and approach described in Zhang, S. *et al.* Gene (1991) 105:61. Synthetic oligonucleotides corresponding to peptides 1-3 of Example 1 were labeled with $K^{32}P$ using polynucleotide kinase and used as probes as described in the standard cloning manual of Maniatis, T. *et al.* Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2nd ed. 1989). A clone hybridizing to all three probes identified from 40 positive clones as hybridizing to all three of the probes was plaque-purified from a screen of 4×10^5 recombinants and subcloned into pBluescript (Stratagene). It contained an insert of 5571 bp.

Double-stranded cDNA in pBluescript was sequenced in both directions by the dideoxy termination method with Sequanase (USB) according to the manufacturer's instructions. The sequencing showed an open reading frame of 4584 base pairs or 1528 amino acids along with a polyadenylation signal at position 5561. The sequence obtained and the deduced amino acid sequence is shown in Figure 1.

Thus, the deduced protein has a molecular mass of 172 kD and a pI of approximately 4.5. The amino acid sequences of the cyanogen bromide fragments of native receptor match perfectly within the deduced amino acid sequence. The open reading frame begins with an ATG that is flanked by the consensus translation initiation sequence GAGATGG for eucaryotic mRNAs as described by Kozak, M. Nucleic Acids Res (1987) 15:8125.

As shown in Figure 1, the deduced amino acid sequence includes a putative signal, shown underlined, preceding the mature N-terminus Asn-Glu-Arg-etc. Eleven repeats (cad1-cad11) are shown in the extracellular region upstream of the membrane domain, shown with the heavy underline, at positions 1406-1427. The end of the 11th repeat is shown with an arrowhead. The positions of the five CNBR fragments are also shown under the complete sequence.

Figure 2 compares the BT-R_1 sequence obtained herein with other members of the cadherin family. Like known cadherins, the external domain of BT-R_1 is highly repetitive and contains 11 repeats (cad1-cad11; see Figure 2 A). The other cadherins compared in Figure 2 B are mouse P cadherin (mP EC1); *Drosophila fat* EC18 (fat EC18) and protocadherin (PC42 EC2), and human intestinal transporter (HPT-1-EC-1). The eleven repeats of the cadherin motif in BT-R_1 (cad1-cad11) are individually aligned with a single motif sequence from each of the other members of the cadherin family. Conserved residues are boxed. The greatest similarity of BT-R_1 to the cadherins is with the extracellular repeats of the cadherin motif of mouse P-cadherin, Drosophila *fat* tumor suppressor and the protocadherins, although homologies are not high (20–40 homology and 30-60 percent similarity). The conserved repeats of BT-R_1 included AXDXD, DXE, DXNDXXP, one glutamic acid residue and two glycine residues (Figure 2 B). Motifs A/VXDXD, DXNDN are the consensus sequences for calcium binding and two such regions are present in a typical cadherin repeat. In all repeats of BT-R_1, the sequence DXNDN is preceded by 8 to 14 hydrophobic amino acids. Similar hydrophobic sequences also have been observed in the cadherins. The length of the hydrophobic stretches suggests that these areas are not transmembrane regions buy that the represent J-sheet structures commonly present in cadherin-like repeats. BT-R^1 contains a putative cytoplasmic domain of 101 amino acids, smaller than vertebrate cadherin cytoplasmic domains (160 amino acids), and shows no homology to any of the cadherin cytoplasmic domains or to cytoplasmic domains of other proteins to which it has been compared in a current sequence data base.

To confirm that the sequenced clone encoded full-length BT-R_1 protein, total mRNA was prepared from midguts of *M. sexta* subjected to Northern blot by hybridization with the antisense 4.8 kb SacI fragment of the BT-R_1 cDNA clone. The Northern blot analysis was conducted by hybridizing to the antisense probe at 420C and 50% formamide, 5 X Denhardt's Reagent, 5 X SSCP and 50 Tg/ml salmon sperm DNA. The filter was then washed two times with 1 X SSC + 0.1% SDS and two times with 0.15 X SSC + 0.1% SDS at 420C. Each wash was roughly 20 minutes. The filter was then exposed to X-ray film for 24 hours. The 4.8 kb probe hybridized to a single 5.6 kb band.

The BT-R_1 clone was translated using rabbit reticulolysate and the resulting translated products were immunoprecipitated with antisera raised against native protein encoded by BT-R_1. For the *in vitro* translation, pBluescript plasmid containing BT-R_1 cDNA was linearized and transcribed with T_3 polymerase (Pharmacia). The translation was conducted according to manufacturer's instructions with nuclease-treated rabbit reticulolysate (Life Technologies, Inc.). After one hour of incubation at 300C, the reaction mixture was combined with an equal volume of SDS buffer or lysed with 50 mM Tris buffer containing 1% NP40 and 250 mM NaCl (pH 8.0) for immunoprecipitation. Preimmune serum was used as a control. Translation and immunoprecipitation products were electrophoresed on a 7.5% SDS-polyacrylamide gel fixed, treated with Enhance (Dupont NEN), dried and exposed to X-ray film for 12 hours.

Two protein bands of approximately 172 kD and 150 kD as determined by SDS-PAGE were obtained; it is postulated that the 150 kD translation product was due to initiation of translation from an internal methionine at amino acid 242. This is consistent with the observations of Kozak, M. Mol Cell Biol (1989) 9:5073.

Thus, both results confirm that a full-length clone was obtained.

Example 3

Recombinant Production and Characteristics of the BT-R_1 Protein

The BT-R_1 cDNA clone was subcloned into the mammalian expression vector pcDNA3 (Invitrogen) and the construct transfected into COS-7 cells. Membranes isolated from the COS-7 transfectants were solubilized, electrophoresed and ligand blotted with ^{125}I-*cryIA(b)* toxin. The cells were harvested 60 hours after transfection, washed with phosphate-buffered saline and lysed by freezing in liquid nitrogen. Cell membranes were prepared by differential centrifugation as described by Elshourbagy, N.A. *et al.* J Biol Chem (1993) 266:3873. Control cells were COS-7 cells transfected with pcDNA3.

The cell membranes (10 Tg) were separated on 7.5% SDS-PAGE blotted to a nylon membrane and blocked with Tris-buffered saline containing 5% nonfat dry milk powder, 5% glycerol and 1% Tween-20. The nylon membrane was then incubated with ^{125}I-*cryIA(b)* toxin

(2×10^5 cpm/ml) for two hours with blocking buffer, dried and exposed to X-ray film at -700C. The labeled toxin bound to a 210 ± 5 kD protein; the 210 kD band was observed only in lanes containing membranes prepared from either *M. sexta* or COS-7 cells transfected with the BT-R_1 cDNA construct containing 4810 bp of cDNA comprising the open reading frame.

The discrepancy between the 210 kD protein expressed and the calculated 172 kD molecular weight is due to glycosylation of the protein; *in vitro* translation of the cDNA clone, as described above, which does not result in glycosylation, does produce the 172 kD protein. To verify this, the COS-7 produced protein was subjected to digestion with N-glycosidase-F by first denaturing the purified protein by boiling in 1% SDS for 5 minutes followed by addition of NP-40 to a final concentration of 1% in the presence of 0.1% SDS, and then incubating the denatured protein in sodium phosphate buffer, pH 8.5 at 370C with N-glycosidase-F for 10 hours. Controls were incubated under the same conditions without enzyme. Digestion products were separated on a 7.5% SDS-PAGE and stained with Coomassie brilliant blue. This glycosidase treatment reduced the molecular weight of BT-R_1 protein from 210 to 190 kD; this indicates N-glycosylation at some of the 16 consensus N-glycosylation sites in the protein. Treatment of BT-R_1 with O-glycosidase and neuraminidase did not alter the mobility of the protein.

In addition, embryonic 293 cells were transfected with the BT-R_1 cDNA clone in pcDNA3 and incubated with the labeled toxin (0.32 nM) in the presence of increasing concentrations (0 to 10^{-6} M) of unlabeled toxin. Nonspecific binding was measured as bound radioactivity in the presence of 1 TM unlabeled toxin. A value for the dissociation constant (K_d) of 1015 pM was determined by Scatchard analysis; this is approximately the same value that was obtained for the natural receptor as described by Vadlamudi, R.K. *et al.* J Biol Chem (1993) (*supra*).

Example 4

Physiological Effect of BT Toxin

on Modified Embryonic 293 Cells

Both unmodified embryonic 293 cells, and 293 cells which have been modified to produce the BT-R_1 receptor as described in Example 3, when cultured *in vitro* form adherent star-shaped clusters. When BT toxin (200 nM) is added to serum-free medium, the clusters round up and release from the plastic surfaces of the culture dish. This effect is also observed under known conditions of cytotoxicity for 293 cells. The foregoing effect is observed only when the cells are cultured in serum-free medium since the toxin binds to serum and would thus be ineffective under conditions where serum is present.

However, in the presence of anti-receptor antisera, this effect of BT toxin is blocked. Also, when serum is added back to a culture of modified E293 cells which has been treated in serum-free conditions with the toxin, the cells revert to their normal star-shaped adherent cluster shapes. This indicates that the effect of the toxin is reversible.

Claims

1. A polynucleotide in purified and isolated form which comprises a nucleotide sequence which encodes a receptor that specifically binds a *Bacillus thuringiensis* (BT) toxin wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1.

2. The polynucleotide of claim 1 wherein said toxin is the *cryIA(b)* toxin of *B. thuringiensis* subsp. *berliner*, or wherein said toxin is a modified form of BT toxin; or wherein said receptor is the BT-R_1 receptor of the tobacco hornworm *Manduca sexta*.

3. A recombinant expression system for expression of a nucleotide sequence encoding a receptor which specifically binds a BT toxin, wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1,

which expression system comprises said encoding nucleotide sequence operably linked to control sequences operable in host cells.

4. Recombinant host cells modified to contain the expression system of claim 3.

5. A method to produce a receptor which binds a BT toxin which method comprises culturing the cells of claim 4 under conditions wherein said receptor is produced; and

optionally recovering said receptor from the culture.

6. The method of claim 5 wherein said receptor is disposed at the surface of said cells, and wherein the cells are optionally recovered from the culture for use in an assay.

7. Cells expressing BT toxin receptor disposed at their surface prepared by the method of claim 6.

8. A method to assess the binding affinity or cytotoxicity of a candidate pesticide for an insect receptor that binds BT toxin which method comprises contacting said candidate with the cells of claim 7 under conditions which permit binding to occur; and

detecting the presence, absence or amount of said candidate bound to said cells, or assessing the cytotoxicity of said candidate with respect to said cells.

9. A receptor which binds BT toxin produced by the method of claim 5.

10. A purified and isolated polynucleotide which comprises a nucleotide sequence complementary to a nucleotide sequence encoding a receptor which binds to BT toxin wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1.

11. A composition which comprises antibodies specifically immunoreactive with a receptor that specifically binds a BT toxin, said receptor encoded by the polynucleotide of claim 1.

12. A method to modify target cells so as to render them susceptible to interaction with a BT toxin which method comprises modifying said cells to contain the expression system of claim 3; and

culturing the cells under conditions wherein said receptor is disposed at the surface of the cells.

13. Cells modified by the method of claim 12.

14. A method to exert a cytotoxic effect on target cells or on a tissue with which said target cells are associated; therein said target cells include, at their surface, a receptor that specifically binds BT toxin, which method comprises contacting said cells with the antibody composition of claim 11, or with an amount of BT toxin effective to interact with said receptor.

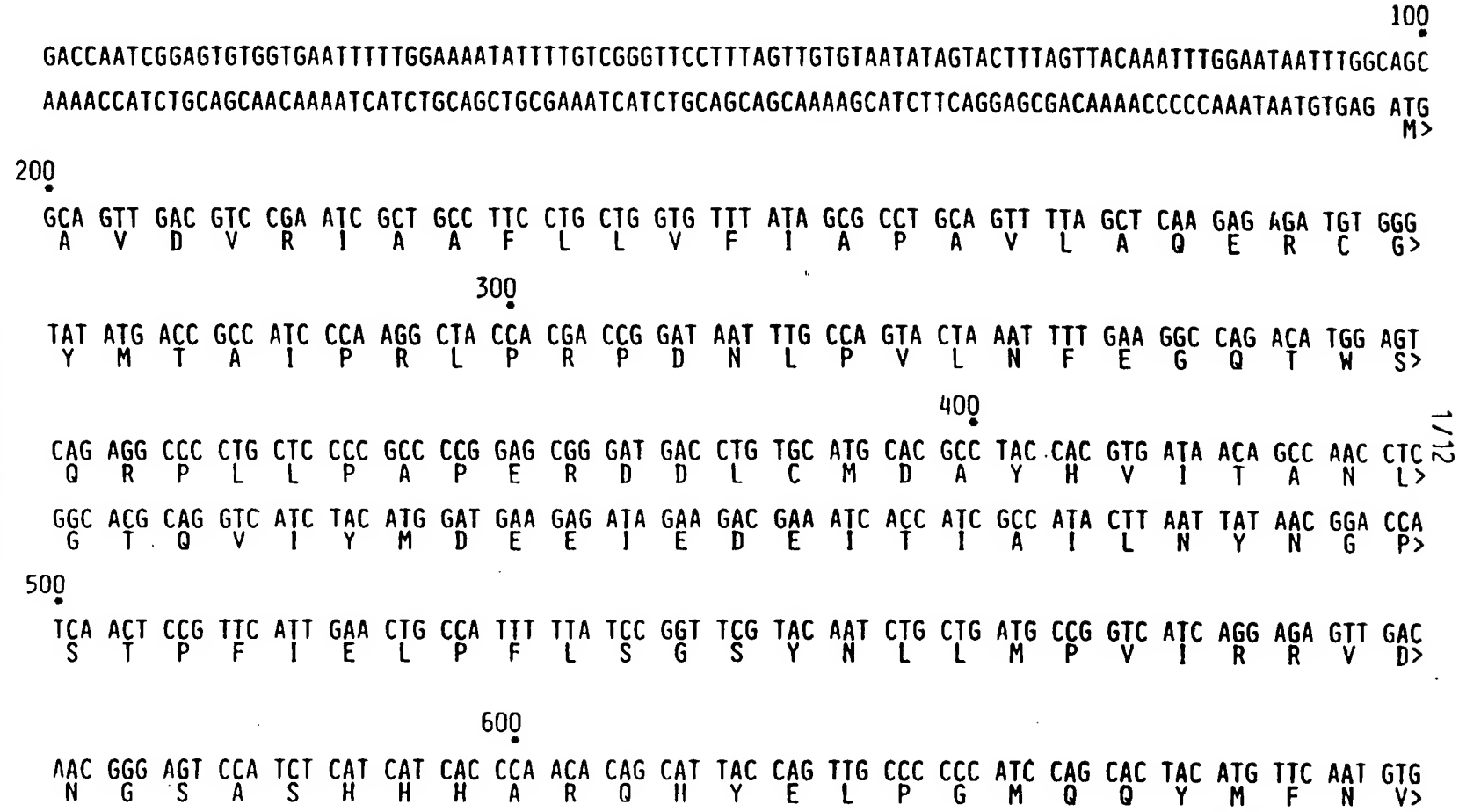

100
GACCAATCGGAGTGTGGTGAATTTTTGGAAAATATTTTGTCGGGTTCCTTTAGTTGTGTAATATAGTACTTTAGTTACAAATTTGGAATAATTTGGCAGC
AAAACCATCTGCAGCAACAAAATCATCTGCAGCTGCGAAATCATCTGCAGCAGCAAAAGCATCTTCAGGAGCGACAAAACCCCCAAATAATGTGAG ATG
M>
200
GCA GTT GAC GTC CGA ATC GCT GCC TTC CTG CTG GTG TTT ATA GCG CCT GCA GTT TTA GCT CAA GAG AGA TGT GGG
A V D V R I A A F L L V F I A P A V L A Q E R C G>
300
TAT ATG ACC GCC ATC CCA AGG CTA CCA CGA CCG GAT AAT TTG CCA GTA CTA AAT TTT GAA GGC CAG ACA TGG AGT
Y M T A I P R L P R P D N L P V L N F E G Q T W S>
400
CAG AGG CCC CTG CTC CCC GCC CCG GAG CGG GAT GAC CTG TGC ATG CAC GCC TAC CAC GTG ATA ACA GCC AAC CTC
Q R P L L P A P E R D D L C M D A Y H V I T A N L>
GGC ACG CAG GTC ATC TAC ATG GAT GAA GAG ATA GAA GAC GAA ATC ACC ATC GCC ATA CTT AAT TAT AAC GGA CCA
G T Q V I Y M D E E I E D E I T I A I L N Y N G P>
500
TCA ACT CCG TTC ATT GAA CTG CCA TTT TTA TCC GGT TCG TAC AAT CTG CTG ATG CCG GTC ATC AGG AGA GTT GAC
S T P F I E L P F L S G S Y N L L M P V I R R V D>
600
AAC GGG AGT CCA TCT CAT CAT CAC CCA ACA CAG CAT TAC CAG TTG CCC CCC ATC CAG CAC TAC ATG TTC AAT GTG
N G S A S H H H A R Q H Y E L P G M Q Q Y M F N V>

FIG. IA

700

CGC GTG GAC GGC CAG TCG CTG GTG GCA GGC GTG TCT CTC GCT ATC GTC AAC ATA GAT GAC AAC GCG CCC ATC ATA
R V D G Q S L V A G V S L A I V N I D D N A P I I>

CAA AAC TTC GAG CCT TGC CGG GTT CCT GAA CTG GGC GAG CCA GGG TTG ACA GAA TGC ACA TAC CAA GTA TCG GAC
Q N F E P C R V P E L G E P G L T E C T Y Q V S D>

800

GCG GAC GGA CGG ATC AGC ACA GAG TTC ATG ACG TTC AGG ATC GAC AGC GTT CGT GGC GAC GAG GAG ACC TTC TAC
A D G R I S T E F M T F R I D S V R G D E E T F Y>

900

ATC GAA CGG ACG AAT ATC CCC AAC CAA TGG ATG TGG CTA AAT ATG ACC ATA GGC GTT AAT ACC TCG CTC AAC TTC
I E R T N I P N Q W M W L N M T I G V N T S L N F>

1000

GTC ACC AGT CCG CTG CAT ATA TTC AGC GTG ACA GCC CTG GAC TCG CTC CCG AAC ACC CAC ACG GTG ACT ATG ATG
V T S P L H I F S V T A L D S L P N T H T V T M M>

GTG CAA GTG GCG AAT GTG AAC AGC CGT CCG CCG CGC TGG CTG GAG ATC TTC GCT GTC CAA CAG TTT GAA GAG AAA
V Q V A N V N S R P P R W L E I F A V Q Q F E E K>

1100

TCT TAC CAA AAC TTC ACA GTG AGG GCG ATC GAC GGA GAC ACT GAG ATC AAT ATG CCT ATC AAC TAC AGG CTG ATC
S Y Q N F T V R A I D G D T E I N M P I N Y R L I>

1200

ACA AAT GAG GAA GAC ACA TTC TTC AGC ATT GAG GCC CTG CCT GGT GGA AAA AGC GGG GCT GTA TTC CTC GTG TCG
T N E E D T F F S I E A L P G G K S G A V F L V S>

FIG. 1B

```
                                                                1300
                                                                 •
CCA ATT GAC CGC GAC ACA CTG CAA CGA GAG GTG TTT CCA CTT ACG ATC GTC GCT TAC AAA TAT GAT GAG GAG GCC
 P   I   D   R   D   T   L   Q   R   E   V   F   P   L   T   I   V   A   Y   K   Y   D   E   E   A>

TTC TCC ACA TCA ACA AAC GTG GTC ATC ATT GTG ACA GAC ATC AAC GAC CAA ACA CCT GAA CCT ATA CAC AAG GAA
 F   S   T   S   T   N   V   V   I   I   V   T   D   I   N   D   Q   R   P   E   P   I   H   K   E>

1400
 •
TAT CGA CTG GCA ATC ATG GAG GAG ACG CCC CTG ACC CTC AAC TTC CAT AAA GAA TTC GGA TTT CAT GAT AAG GAT
 Y   R   L   A   I   M   E   E   T   P   L   T   L   N   F   D   K   E   F   G   F   H   D   K   D>

                                1500
                                 •
TTA GGT CAA AAC GCT CAG TAC ACG GTG CGT CTA GAG AGC GTG GAC CCT CCA CCC GCT GCT GAG GCA TTC TAC ATA
 L   G   Q   N   A   Q   Y   T   V   R   L   E   S   V   D   P   P   G   A   A   E   A   F   Y   I>

                                                                1600
                                                                 •
GCG CCT GAA GTC GGC TAC CAG CGA CAG ACC TTC ATC ATG CCC ACC CTC AAT CAC TCC ATG CTG GAT TAC GAA GTG
 A   P   E   V   G   Y   Q   R   Q   T   F   I   M   G   T   L   N   H   S   M   L   D   Y   E   V>

CCA GAG TTT CAG AGT ATT ACG ATT CGG GTG GTA GCG ACC GAC AAC AAC GAC ACG AGG CAC GTG GGC GTC GCG TTG
 P   E   F   Q   S   I   T   I   R   V   V   A   T   D   N   N   D   T   R   H   V   G   V   A   L>

1700
 •
GTT CAC ATT CAC CTC ATC AAT TGG AAC GAT GAG CAG CCG ATC TTC GAA CAC GCC GTG CAG ACC GTC ACC TTC GAC
 V   H   I   D   L   I   N   W   N   D   E   Q   P   I   F   E   H   A   V   Q   T   V   T   F   D>

                                1800
                                 •
GAG ACT GAA GCC GAG GGG TTC TTC GTC GCC AAG GCG GTT GCA CAC GAC AGA GAC ATC GGG GAT GTC GTC GAG CAT
 E   T   E   G   E   G   F   F   V   A   K   A   V   A   H   D   R   D   I   G   D   V   V   E   H>
```

FIG. IC

1900

ACT TTA TTG GGT AAC GCT GTT AAC TTC CTG ACC ATC GAC AAA CTC ACC GGC GAC ATC CGC GTC TCA GCT AAC GAC
T L L G N A V N F L T I D K L T G D I R V S A N D>

TCC TTC AAC TAC CAT CGA GAA AGT GAA TTA TTT GTG CAG GTG CGA GCT ACA GAC ACG CTG GGG CAA CCC TTC CAC
S F N Y H R E S E L F V Q V R A T D T L G Q P F H>

2000

ACG GCG ACG TCA CAG CTG GTC ATA CGA CTA AAT GAC ATC AAC AAC ACG CCA CCC ACC TTA CGG CTG CCT CGA GGC
T A T S Q L V I R L N D I N N T P P T L R L P R G>

2100

AGT CCC CAA GTG GAG GAG AAC GTG CCT GAT GCG CAC GTC ATC ACC CAG GAG TTA CGC GCC ACC GAC CCC GAC ACC
S P Q V E E N V P D A H V I T Q E L R A T D P D T>

2200

ACG GCC GAT CTG CGC TTC GAG ATA AAC TGG GAC ACC TCT TTC GCC ACC AAG CAA GGC CGC CAG GCT AAC CCC GAC
T A D L R F E I N W D T S F A T K Q G R Q A N P D>

GAG TTT AGG AAT TGC GTG GAA ATC GAG ACC ATC TTC CCC GAG ATT AAC AAC CGG GGA CTG GCT ATC GGC CGC GTT
E F R N C V E I E T I F P E I N N R G L A I G R V>

2300

GTA GCG CGC GAA ATC AGA CAC AAC GTG ACC ATA GAC TAC GAG GAG TTT GAG GTC CTC TCC CTC ACA GTG AGG GTG
V A R E I R H N V T I D Y E E F E V L S L T V R V>

2400

CGT GAC CTT AAC ACC GTC TAC GGA GAC GAC TAC GAC GAA TCG ATG CTC ACA ATA ACT ATA ATC CAT ATG AAC GAC
R D L N T V Y G D D Y D E S M L T I T I I D M N D>

FIG. ID

2500

AAC GCG CCG GTG TGG GTG GAG GGG ACT CTG GAG CAG AAC TTC CGA GTC CGC CAG ATG TCG GCG GGC GGG CTC GTG
N A P V W V E G T L E Q N F R V R E M S A G G L V>

GTG GGC TCC GTG CGC GCG GAC GAC ATC GAC GGA CCG CTC TAC AAC CAA GTG CGA TAC ACC ATT TTC CCT CGT GAA
V G S V R A D D I D G P L Y N Q V R Y T I F P R E>

2600

GAC ACA GAT AAG GAC CTG ATA ATG ATC GAA CTT CCT CAC GGG TCA AAT TTC CGT GAA CAC AAG CGG CGC ATC GAC
D T D K D L I M I E L P H G S N F R E H K R R I D>

2700

GCG AAT ACT CCT CCA CGC TTC CAC CTC TAC TAT ACA GTG GTC GCT AGT GAC CGA TGC TCG ACA GAA GAT CCT GCA
A N T P P R F H L Y Y T V V A S D R C S T E D P A>

2800

GAT TGC CCC CCT GAC CCG ACT TAT TGG GAA ACC GAA GGA AAT ATC ACA ATC CAC ATC ACC GAC ACG AAC AAC AAG
D C P P D P T Y W E T E G N I T I H I T D T N N K>

GTC CCG CAG GCG CAA ACG ACT AAG TTC GAT ACC GTC GTG TAT ATT TAC GAG AAC GCA ACC CAC TTA GAC GAG GTG
V P Q A E T T K F D T V V Y I Y E N A T H L D E V>

2900

GTC ACT CTG ATA GCC AGT GAT CTT GAC AGA GAC GAA ATA TAC CAC ATG GTG AGC TAC GTC ATC AAT TAT GCA GTG
V T L I A S D L D R D E I Y H M V S Y V I N Y A V>

3000

AAC CCT CGA CTG ATG AAC TTC TTC TCC GTG AAC CGA GAG ACC GGC CTG GTG TAC GTG GAC TAT GAG ACC CAG GTT
N P R L M N F F S V N R E T G L V Y V D Y E T Q G>

FIG. IE

3100

AGT GGC CAG GTG CTG CAC CGT CAT GGT GAT GAA CCA ACG CAC CGT ATC TTC TTC AAC CTC ATC GAC AAC TTC ATG
S G E V L D R D G D E P T H R I F F N L I D N F M>

GGG CAA GCA GAA GGT AAC AGA AAT CAG AAC GAC ACA GAA GTT CTC GTT ATC TTG TTG GAT GTG AAT GAC AAT GCT
G E G E G N R N Q N D T E V L V I L L D V N D N A>

3200

CCT GAA TTG CCA CCG CCG AGC GAA CTC TCT TGG ACT ATA TCT GAG AAC CTT AAG CAG GGC GTC CGT CTT GAA CAA
P E L P P P S E L S W T I S E N L K Q G V R L E P>

3300

CAT ATC TTC GCC CCG GAC CGC GAC GAG CCC GAC ACA CAC AAC TCC AGG GTC GGC TAC GAG ATC CTG AAC CTC AGC
H I F A P D R D E P D T D N S R V G Y E I L N L S>

3400

ACG GAG CGG GAC ATC GAA GTG CCG GAC CTC TTT GTG ATG ATA CAG ATC GCG AAC GTC ACG GGA GAG CTG GAG ACC
T E R D I E V P E L F V M I Q I A N V T G E L E T>

GCC ATG GAC CTC AAG GGA TAT TGG GGG ACG TAC GCT ATA TAT ATA CTG GCA TTC GAC CAC GGC ATT CCG CAA ATG
A M D L K G Y W G T Y A I Y I L A F D H G I P Q M>

3500

TCC ATG AAC GAG ACA TAT GAG CTG ATC ATC CAT CCG TTC AAC TAC TAC GCG CCT GAG TTC GTC TTC CCG ACC AAC
S M N E T Y E L I I H P F N Y Y A P E F V F P T N>

3600

GAT GCC GTC ATA CGA CTT GCG AGG GAA CGA GCT GTA ATC AAT GGA GTT GTA GCG ACA GTG AAC GGA GAG TTC TTG
D A V I R L A R E R A V I N G V L A T V N G E F L>

FIG. 1F

3700

GAG CGG ATA TCG GCG ACT CAT CCG GAC GGA CTC CAC GCG GGC GTC GTC ACC TTC CAA GTG GTA GGC GAT GAG GAA
E R I S A T D P D G L H A G V V T F Q V V G D E E>

TCA CAA CGG TAC TTT CAA GTA GTT AAC GAT GGC GAG AAC CTC GGC TCG TTG AGG TTA CTG CAA CCC GTT CCA GAG
S Q R Y F Q V V N D G E N L G S L R L L Q A V P E>

3800

GAG ATC AGG GAG TTC CGG ATA ACG ATT CGC GCT ACA GAC CAG GGA ACG GAC CCA GGA CCG CTG TCC ACG GAC ATG
E I R E F R I T I R A T D Q G T D P G P L S T D M>

3900

ACG TTC AGA GTT GTT TTT GTG CCC ACG CAA GGA GAA CCT AGA TTC GCG TCC TCA GAA CAT GCT GTC GCT TTC ATA
T F R V V F V P T Q G E P R F A S S E H A V A F I>

4000

GAA AAG AGT GCC GGC ATG GAA GAG TCT CAC CAA CTT CCT CTA GCT CAA GAC ATC AAG AAC CAT CTC TGT GAA GAC
E K S A G M E E S H Q L P L A Q D I K N H L C E D>

GAC TGT CAC AGC ATT TAC TAT CGT ATT ATC GAC GGC AAC AGC GAA GGT CAT TTC GGC CTG GAT CCT GTT CGC AAC
D C H S I Y Y R I I D G N S E G H F G L D P V R N>

4100

AGG TTG TTC CTG AAG AAA GAG CTG ATA AGG GAA CAA AGT GCC TCC CAC ACT CTG CAA GTG GCG GCT AGT AAC TCG
R L F L K K E L I R E Q S A S H T L Q V A A S N S>

4200

CCC GAT GGT GGC ATT CCA CTT CCT GCT TCC ATC CTT ACT GTC ACT GTT ACC GTG AGG GAG GCA GAC CCT CGT CCA
P D G G I P L P A S I L T V T V T V R E A D P R P>

FIG. 1G

```
                                                                4300
GTG TTT GTG AGG CAA TTC TAC ACC GCA GGG ATA TCC ACA GCG GAC TCC ATC GGC AGA GAG CTG CTC AGA TTA CAT
 V   F   V   R   E   L   Y   T   A   G   I   S   T   A   D   S   I   G   R   E   L   L   R   L   H>

GCG ACC CAC TCT CAA GGC TCG CCC ATT ACT TAT GCT ATA GAC TAC GAT ACA ATG GTA GTG GAC CCC AGC CTG GAG
 A   T   Q   S   E   C   S   A   I   T   Y   A   I   D   Y   D   T   M   V   V   D   P   S   L   E>

4400
GCA GTG AGA CAG TCG GCT TTC GTA CTG AAC GCT CAA ACC GGA GTG CTG ACG CTT AAT ATC CAG CCC ACG GCC ACG
 A   V   R   Q   S   A   F   V   L   N   A   Q   T   G   V   L   T   L   N   I   Q   P   T   A   T>

                                4500
ATG CAT GGA CTG TTC AAA TTC GAA GTC ACA GCT ACT GAC ACG GCC GGC GCT CAG GAC CGC ACC GAC GTC ACC GTG
 M   H   G   L   F   K   F   E   V   T   A   T   D   T   A   G   A   Q   D   R   T   D   V   T   V>

                                                                4600
TAC GTG GTA TCC TCG CAG AAC CGC GTC TAC TTC GTG TTC GTC AAC ACG CTG CAA CAG GTC GAA CAC AAC AGA GAC
 Y   V   V   S   S   Q   N   R   V   Y   F   V   F   V   N   T   L   Q   Q   V   E   D   N   R   D>

TTT ATC GCG GAC ACC TTC AGC GCT GGG TTC AAC ATG ACC TGC AAC ATC GAC CAA GTG GTG CCC GCT AAC GAC CCC
 F   I   A   D   T   F   S   A   G   F   N   M   T   C   N   I   D   Q   V   V   P   A   N   D   P>

4700
GTC ACC GGC GTG GCG CTG GAG CAC AGC ACG CAG ATG GCG GCC ACT TCA TAC GGG ACA ACG TAC CCG TAC TCG CTG
 V   T   G   V   A   L   E   H   S   T   Q   M   A   A   T   S   Y   G   T   T   Y   P   Y   S   L>

                                4800
ATG AGA TAGAACAGATCCGTAGTGACCTAGTCCTCCTGAGCTCCATACAAACAACGCTGGCTGGCGCGATCGTGGTGTTGCAGGACTTGTTGACCAAC
 M   R>
```

FIG. IH

4900

TCCAGCCCGGACTGTCTGACTCGAGCCTCACGGTGTACGTCTGCCTCACTGTCTGCTGTCTCGGTTTCATGTGCCTTGTGCTACTGCTTACCTTCATCAT

5000

CAGGACTAGAGCGCTAAACCGACGGTTGGAAGCCCTGTCGATGACGAAGTGAGGGCTCACTGGACTCTGGATTGAACCGCGCCGGCATCGCCGCCCCCGG

5100

CACCAACAAACACACTGTGGAAGGCTCCAACCCTATCTTCAATGAACCAATAAAGACGCCAGATTTAGATGCCATTAGCGAGGGTTCCAACGACTCTGAT

5200

CTGATCGGCATCGAAGATCTTCCCACTTTGGCAACCACTTCATGGATCCTGAGGTGAACGAAAAGGCAAATCCTTATCCCGAAGTCGCAAACCACAACAA

5300

CAACTTCGGCTTTCAACCGACTCCCTTCTCGCCTGAGTTCGTTAACGGACAGTTCAGAAAGATCTAGAAGATAACAACACTAGTTAAGATCATTAATTTT

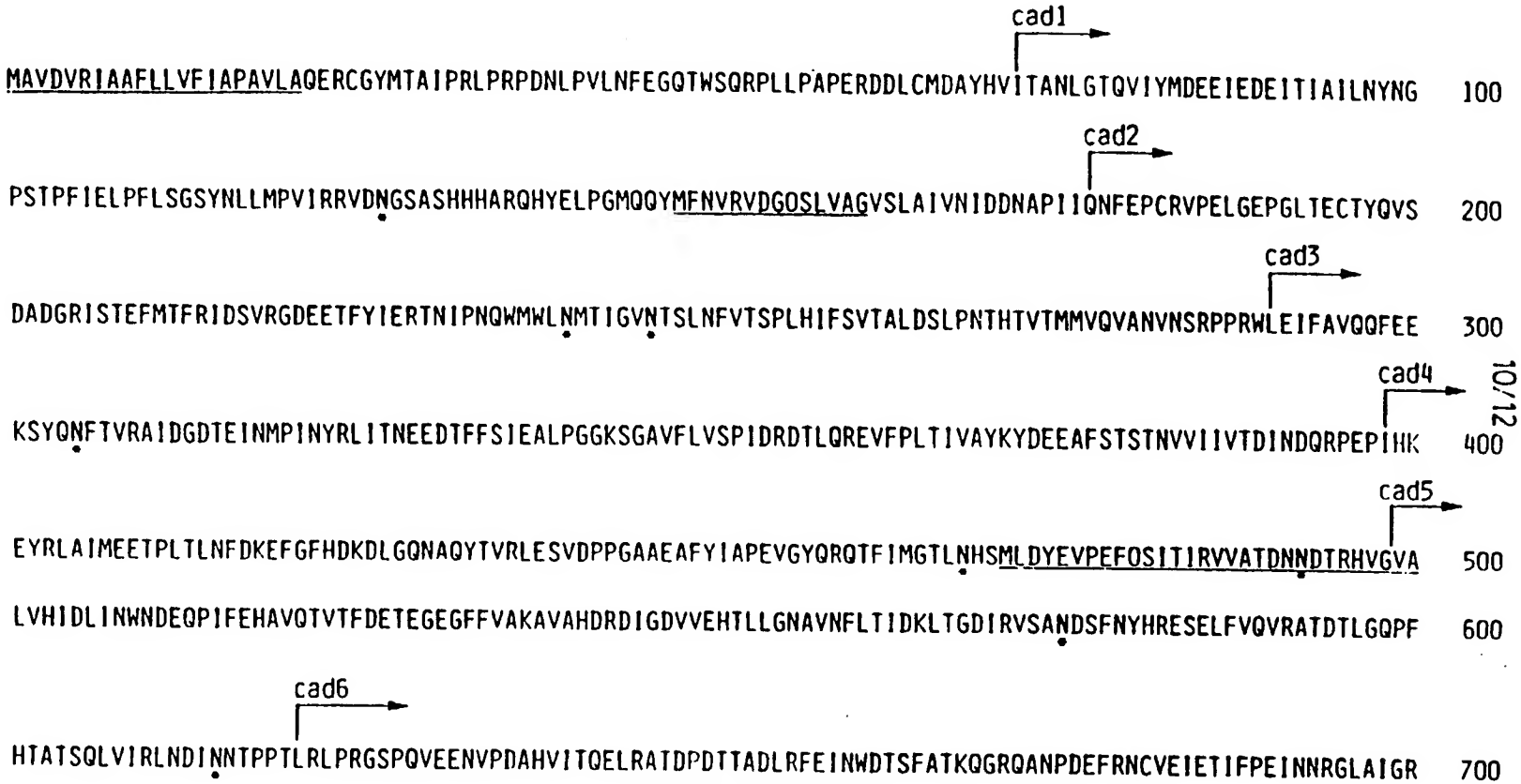
5400

GGAGTTTGGAATTAAGATTTTTGAAAGCATACTTGTGATAAGAATGTGATTTTTAAAACTGTAATTCAAAAAAAAAATTGAGACCTCCATTTAAGCTCTT

5500

GCTCTCATCTCATCAAATTTTATAAAATGCCATTAGTCATTAAGATACTCGATTTAATTTAACATTATTTAAGATATTATGTAAAATAAATATATTGTC

FIG. II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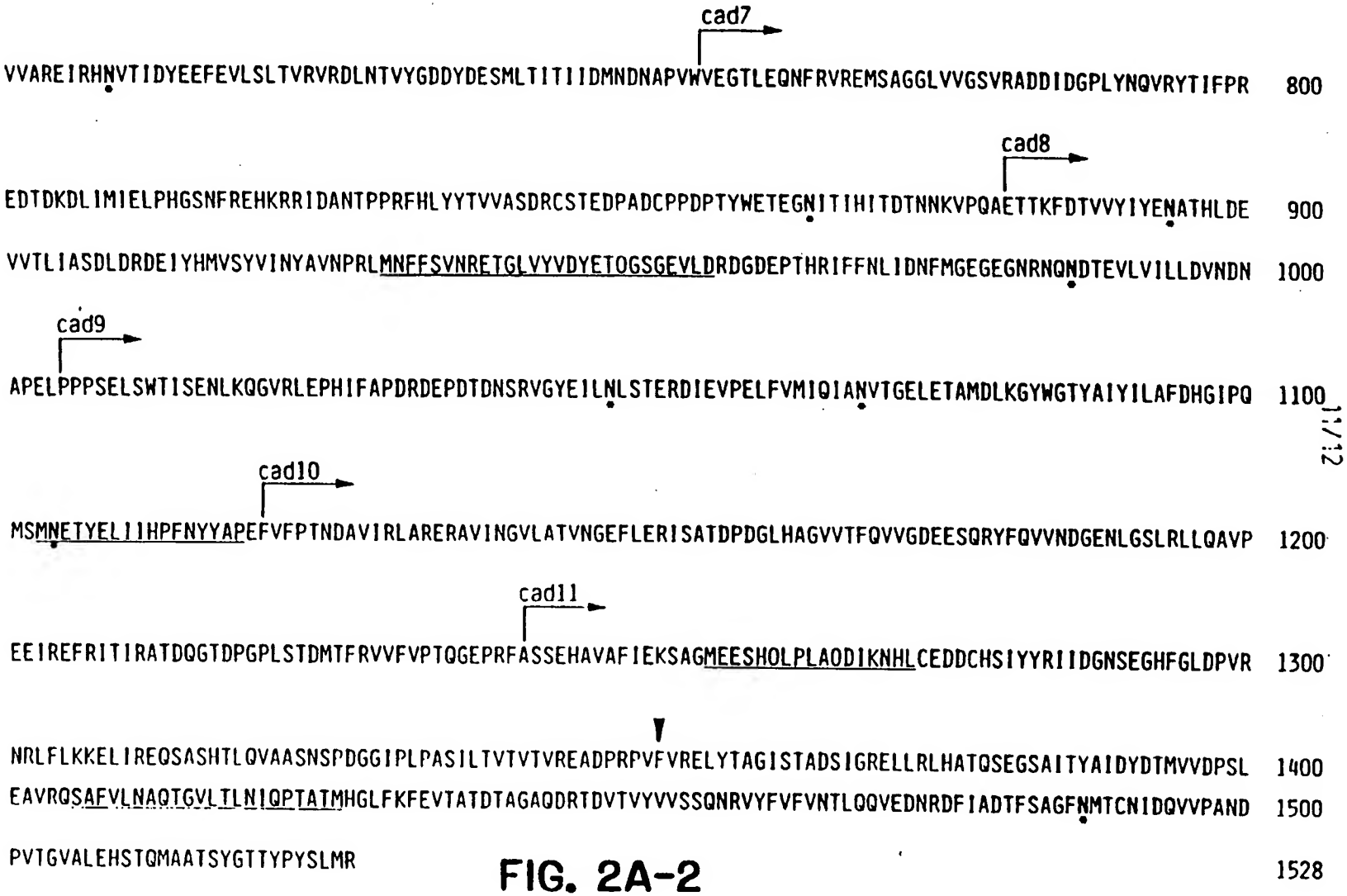
cad2
PSTPFIELPFLSGSYNLLMPVIRRVDNGSASHHHARQHYELPGMQQYMFNVRVDGQSLVAGVSLAIVNIDDNAPIIQNFEPCRVPELGEPGLTECTYQVS 200
cad3
DADGRISTEFMTFRIDSVRGDEETFYIERTNIPNQWMWLNMTIGVNTSLNFVTSPLHIFSVTALDSLPNTHTVTMMVQVANVNSRPPRWLEIFAVQQFEE 300
cad4
KSYQNFTVRAIDGDTEINMPINYRLITNEEDTFFSIEALPGGKSGAVFLVSPIDRDTLQREVFPLTIVAYKYDEEAFSTSTNVVIIVTDINDQRPEPIHK 400
cad5
EYRLAIMEETPLTLNFDKEFGFHDKDLGQNAQYTVRLESVDPPGAAEAFYIAPEVGYQRQTFIMGTLNHSMLDYEVPEFQSITIRVVATDNNDTRHVGVA 500
LVHIDLINWNDEQPIFEHAVQTVTFDETEGEGFFVAKAVAHDRDIGDVVEHTLLGNAVNFLTIDKLTGDIRVSANDSFNYHRESELFVQVRATDTLGQPF 600
cad6
HTATSQLVIRLNDINNTPPTLRLPRGSPQVEENVPDAHVITQELRATDPDTTADLRFEINWDTSFATKQGRQANPDEFRNCVEIETIFPEINNRGLAIGR 700

FIG. 2A-1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cad8
EDTDKDLIMIELPHGSNFREHKRRIDANTPPRFHLYYTVVASDRCSTEDPADCPPDPTYWETEGNITIHITDTNNKVPQAETTKFDTVVYIYENATHLDE 900
VVTLIASDLDRDEIYHMVSYVINYAVNPRLMNFFSVNRETGLVYVDYETQGSGEVLDRDGDEPTHRIFFNLIDNFMGEGEGNRNQNDTEVLVILLDVNDN 1000
cad9
APELPPPSELSWTISENLKQGVRLEPHIFAPDRDEPDTDNSRVGYEILNLSTERDIEVPELFVMIQIANVTGELETAMDLKGYWGTYAIYILAFDHGIPQ 1100
cad10
MSMNETYELIIHPFNYYAPEFVFPTNDAVIRLARERAVINGVLATVNGEFLERISATDPDGLHAGVVTFQVVGDEESQRYFQVVNDGENLGSLRLLQAVP 1200
cad11
EEIREFRITIRATDQGTDPGPLSTDMTFRVVFVPTQGEPRFASSEHAVAFIEKSAGMEESHQLPLAQDIKNHLCEDDCHSIYYRIIDGNSEGHFGLDPVR 1300
NRLFLKKELIREQSASHTLQVAASNSPDGGIPLPASILTVTVTVREADPRPVFVRELYTAGISTADSIGRELLRLHATQSEGSAITYAIDYDTMVVDPSL 1400
EAVRQSAFVLNAQTGVLTLNIQPTATMHGLFKFEVTATDTAGAQDRTDVTVYVVSSQNRVYFVFVNTLQQVEDNRDFIADTFSAGFNMTCNIDQVVPAND 1500
PVTGVALEHSTQMAATSYGTTYPYSLMR 1528

FIG. 2A-2

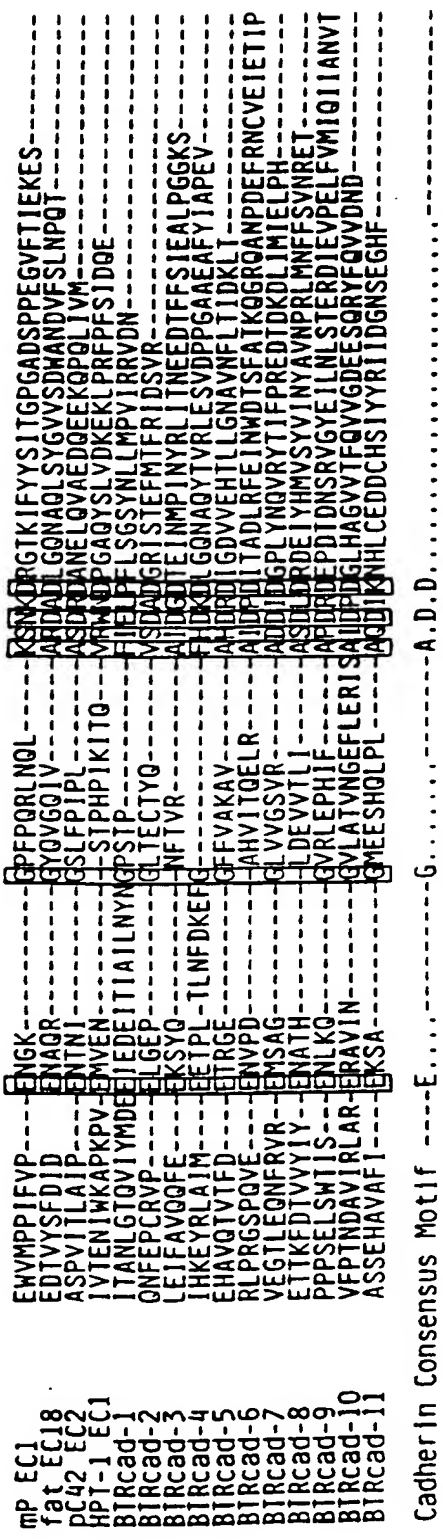

FIG. 2B-1

FIG. 2B-2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13256

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :536/23.5; 435/7.21, 69.1, 240.2; 530/387.1; 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/7.21, 69.1, 240.2; 530/387.1; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, AGRICOLA, EMBASE, MEDLINE, DERWENT, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 268, No. 17, issued 15 June 1993, VADLAMUDI et al, "A Specific Binding Protein from Manduca sexta for the Insecticidal Toxin of Bacillus thuringiensis subsp. berliner", pages 12334-12340, see the entire document.	1-14
X, P	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 270, No. 10, issued 10 March 1995, VADLAMUDI et al, "Cloning and Expression of a Receptor for an Insecticidal Toxin of Bacillus thuringiensis", pages 5490-5494, see the entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 JANUARY 1996	Date of mailing of the international search report 13 FEB 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRUCE CAMPELL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13256

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/567; C12P 21/06; C12N 5/00; A01N 37/18; C07K 16/00; C07H 21/04